

Proteolytic autodigestion

Common tissue pathology in Shwachman–Diamond syndrome?

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Disease modeling using induced pluripotent stem cells (iPSCs) and human embryonic stem cells (hESCs) promises to reveal underlying pathophysiology, especially for genetic diseases that manifest pathologic tissue development, or in cases where disease tissues are largely destroyed by disease at the time of diagnosis. Shwachman–Diamond syndrome (SDS), a rare pediatric disease marked by neutropenia (bone marrow failure) and exocrine pancreatic insufficiency, is one such condition. Given its low prevalence and the need for invasive procedures in affected children to acquire pancreatic or bone marrow samples, obtaining primary diseased tissues is challenging. The availability of iPSCs from affected patients, however, affords the opportunity to revisit hematopoietic and pancreatic tissue development in vitro, with scientists holding a ringside seat.

SDS is associated with mutations in the Shwachman–Bodian–Diamond syndrome (SBDS) gene,¹ which encodes a protein involved in 60S ribosomal subunit maturation²; however, why a general ribosomal assembly defect should manifest as pathology within the pancreatic and hematopoietic lineages has remained a mystery. Our laboratory recently addressed this question through disease modeling of SDS with iPSCs and hESCs.³ We reprogrammed iPSCs from patients with SDS^{4,5} as well as knocked down the *SBDS* gene using short hairpin inhibitory RNAs.³ We then rescued these cells with lentiviral

vectors carrying either normal *SBDS* or an empty vector to generate appropriately matched gene-corrected control lines. We found that, once differentiated into target tissues, iPSCs/hESCs with SDS protein deficiency but not gene-corrected control cell lines recapitulated the pancreatic, hematopoietic, and ribosomal defects observed in patients.³ These findings demonstrated that our iPSC/hESC differentiated tissues appropriately model SDS in vitro.

By analysis of cellular morphology and various biochemical features of the cell cultures, we found that both pancreatic and hematopoietic cells differentiated from SBDS-deficient iPSC/hESC cell lines expressed larger and greater numbers of pancreatic zymogenic and myeloid azurophilic granules, higher protease levels, and more prominent regions of cell death compared with gene-corrected controls. Interestingly, incubation with aprotinin or cocktails of anti-proteolytic compounds during differentiation increased cell survival and restored normal morphology in cultures,³ leading us to hypothesize that increased protease levels predispose these highly granule-laden cells to auto-digestion, thereby providing a common pathogenic mechanism to explain the defects in pancreas and bone marrow cells from SDS patients. We speculate that, when stimulated, these abnormal granules release activated proteases either intracellularly or in the proximal extracellular space, resulting in auto-digestion of the cells and tissue

destruction. Consequently, cell death of pancreatic acinar cells or early myeloid cells, both of which have naturally high numbers of granules, results in functional tissue deficiency.

Anti-proteolytic compounds may offer a novel therapeutic option for patients with SDS, perhaps for patients experiencing acute exacerbations of neutropenia and infection. While the previously FDA-approved compound aprotinin (Trasylol™) was withdrawn from the market, newer anti-proteolytic compounds have been FDA-approved, such as the kallikrein inhibitor ecallantide (Kalbitor™), or are under development, such as the neutrophil elastase inhibitor depelestat, making them attractive options for investigation. We postulate that exocrine pancreatic cells differentiated from SDS patient-derived iPSC will provide a platform for screening the efficacy of anti-proteolytic compounds in this disease. Any compound successful in a broad iPSC-based drug screen could then be introduced into an animal model, such as a conditional knockout mouse that replicates the phenotype of SDS,⁶ and, if promising, translated into a pilot clinical trial. A parallel approach for identifying therapeutic compounds will be especially helpful in the hematopoietic compartment, as infection secondary to neutropenia is a major cause of mortality in patients with SDS.

Using stem cell-derived tissues from SDS patients will also allow investigations into ribosome biology. A fully functioning

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ribosome requires a complex formed between the 40S subunit, the 60S subunit, and mRNA, assisted by numerous accessory proteins. SBDS participates in the release of an accessory protein, eukaryotic initiation factor 6 (eIF6) from the pre-60S subunit, thereby allowing its binding with the 40S/mRNA complex. Therefore, iPSC cultures from patients with SDS model a defect in a 60S subunit maturation. Many other diseases occur due to other faulty steps in ribosomal biogenesis. For example, Diamond Blackfan anemia (DBA) can result from defects in 40S subunit maturation. A recently reported iPSC model of DBA⁷ along with our iPSC/hESC model of SDS may prove valuable in teasing out

the mechanisms underlying ribosomal maturation, in describing the pathogenesis of when maturation fails, and in testing compounds or stimuli that can alter or correct maturation defects. Our laboratory has likewise recently generated iPSC from Pearson marrow pancreas syndrome, a mitochondrial disorder, to investigate basic mitochondrial biology, providing an example of using stem cell technologies to interrogate organelle function and involvement in disease.⁸

iPSCs and hESCs demonstrate significant potential in revealing the pathogenesis of human disease and for testing pharmaceutical therapies. We anticipate many more insights to emerge from this “disease-in-a-dish” approach.

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